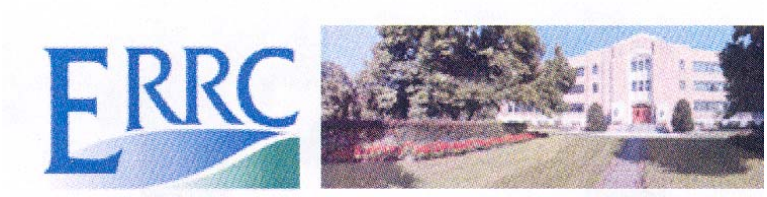

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Title: Radiation Sensitivity of a Pathogen *(Listeria monocytogenes)* and a Surrogate *(Listeria innocua)* on Inoculated Endive *(Cichorium endiva)*

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Report #1: Radiation Sensitivity of a Pathogen *(Listeria monocytogenes)* and a Surrogate *(L. innocua)* on Inoculated Endive (*Cichorium endiva*)

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ABSTRACT

Leaf pieces and leaf homogenate of endive (*Cichorium endiva*) were inoculated with the pathogen *Listeria monocytogenes* or *Listeria innocua*, a non-pathogenic surrogate bacterium. The radiation sensitivity of the two strains were found to be similar, although *L. innocua* was more sensitive to the type of suspending leaf preparation. During refrigerated storage following irradiation, the population of *L. monocytogenes* on inoculated endive was briefly suppressed by 0.42 kGy, a dose calibrated to achieve a 99% reduction. However, the pathogen regrew after 5 days until it exceeded the bacterial levels on the control after 19 days in storage. Treatment with 0.84 kGy, equivalent to 99.99% reduction, suppressed *L. monocytogenes* throughout the course of refrigerated storage. Doses up to 1.0 kGy had no significant effect on color of endive leaf material, whether taken from the leaf edge or the leaf midrib. The texture of leaf edge material was unaffected by doses up to 1.0 kGy, while the maximum dose tolerated by leaf midrib material was 0.8 kGy. These results show that endive leaves may be treated with doses sufficient to achieve at least a 99.99% reduction of *L. monocytogenes* with little or no impact on the product's texture or color.

OBJECTIVES

The objectives of this study were to determine 1) the radiation sensitivity of a pathogen, *L. monocytogenes* (*L.m.*), and a commonly used surrogate, *L. innocua*, when inoculated onto endive (*Cichorium endiva*), a leafy salad vegetable, 2) the survival and potential for regrowth of *L. monocytogenes* on irradiated, stored endive, and 3) the effect of efficacious doses of radiation on the texture and color of endive leaves.

MATERIALS AND METHODS

1) Product: Sanitized endive leaf pieces, leaf homogenates

2) Microorganisms: *L. m.* (ATCC 49594), *L. innocua* (ATCC 51742), grown in TSB.

3) Inoculation: Leaf homogenate was inoculated 100:1 with either *L. m.* or *L. innocua* culture. Cut leaf pieces were inoculated by submersion in 1000 ml of either inoculum in a biosafety hood (to avoid aerosolization of bacteria). Leaf material was dried in a salad spinner to remove excess inoculum. Leaf material was inoculated and bagged for D_{10} determination and, separately, for storage survival and recovery. Each experiment was performed three

4) Irradiation: Temperature controlled Cs-137 gamma source, 0.098 kGy/min. Dosimetry was alanine pellets/EPR, dose delivered at 2°C. Doses for D_{10} values: leaf homogenate - 0.0 (control), 0.2, 0.4, 0.6, 0.8 or 1.0 kGy; cut leaf pieces - 0.0 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.75 or 1.0 kGy. Dose for storage: 0.0 (control), 0.42 or 0.84 kGy.

5) Sampling: Leaf pieces were surface-washed with sterile BPB, serially diluted. Homogenates were serially diluted. Diluted samples pour plated with TSA. D_{10} calculated from log reduction curve. Following storage, diluted samples were plated on TSA and Palcam to separate *L. m.* from TAPC.

6) Sensory properties: Cut leaf pieces treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8 or 1.0 kGy as described, and held at 2EC until sampling, typically 90-120 min. The study was performed three times, with samples irradiated concurrently. Color values were taken with a Hunter Lab Miniscan XE meter to determine the brightness, greenness/redness and blueness/yellowness of the material. The maximum shear strength of the leaf sections was measured with a TA.XT2i texture analyzer using a TA-91 Kramer Shear Press with 5 blades.

RESULTS

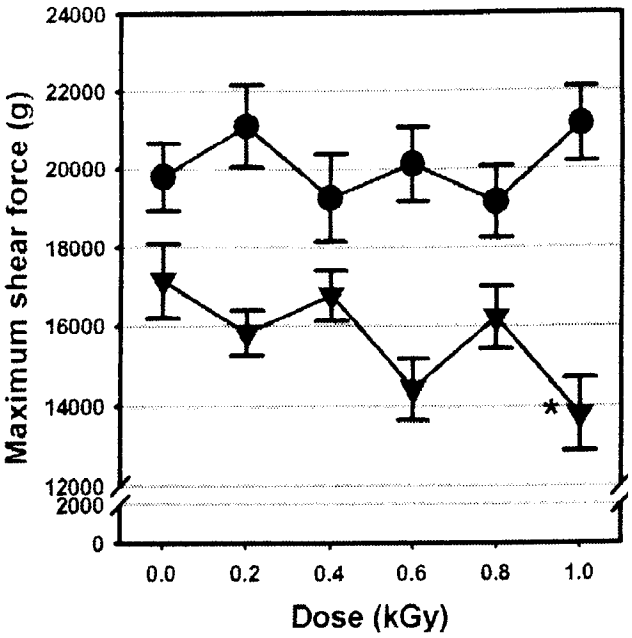
A.) D_{10} values: Irradiation effectively reduced the population of *L. m.* and *L. innocua.* The D_{10} values obtained did not differ significantly ($P<0.05$) between *L. m.* and *L. innocua* on either leaf homogenates (0.20 vs. 0.19 kGy, respectively) or leaf pieces (0.21 vs. 0.22 kGy, respectively). However, the D_{10} for *L. innocua* was significantly ($P<0.05$) lower on leaf homogenates vs. leaf pieces (0.19 vs. 0.22 kGy), while the D_{10} for *L. m.* was not sensitive to leaf preparation method.

B.) Storage and regrowth: A dose of 0.42 or 0.84 kGy reduced the initial population of *L. m.* by 2.6 or 4.0 $\log_{10}$. The *L. m.* population was slightly reduced on the untreated controls after two days of refrigerated storage, but remained stable thereafter through the 19 days of the study. Following 0.42 kGy, the population similarly declined slightly until five days, then rebounded and increased at the 14-day period. At the final sampling time, 19 days, the population of *L. m.* was slightly (0.4 $\log_{10}$ units) greater than on the control, a statistically significant difference ($P<0.05$). Following 0.84 kGy, the levels of *L. m.* increased slightly during storage, although the population remained significantly lower than the control throughout the course of the study. TAPC was generally 0.5 to 1.0 $\log_{10}$ units greater than counts on Palcam agar at the comparable dose-time combinations. The behavior of the TAPC in response to irradiation was generally similar to that of the *L. m.* population. TAPC increased on samples treated with 0.42 kGy until, at day 14 and 19, they were not significantly different from the controls. TAPC was reduced following 0.84 kGy, but by days 14 and 19, the counts were significantly higher than immediately after treatment.

C.) Sensory: Irradiation up to 1.0 kGy had no significant effect on the color of leaf tissue. Material taken from the leaf edge was generally darker and greener than material taken from the leaf midrib.

For leaf material taken from the leaf edge (circle, in Graph #1, following), irradiation doses up to 1.0 kGy had no effect on texture, with the maximum shear force obtained at each dose being not statistically different (ANOVA, $P<0.05$) from the control.

Material taken from the leaf midrib was similarly insensitive to doses up to 0.8 kGy (triangle, in Graph #1); however, at the highest dose examined, 1.0 kGy, the maximum shear force was significantly less (ANOVA, $P<0.05$) than that of the control.



CONCLUSIONS

This study has shown that on a leafy green vegetable, *L. innocua* ATCC 51742 has a similar response to *L. monocytogenes* ATCC 49594 and may therefore be regarded as a valid surrogate model organism on this product for evaluations of radiation sensitivity. Following very low radiation doses, equivalent to 2 D_{10} units, an initial decline in *L. monocytogenes* population was fully recovered by 19 days in storage. Higher doses result in a more lasting suppression of *L. monocytogenes* on stored endive, and these doses (0.8 -1.0 kGy) have little or no significant impact on the product's sensorial properties. In designing protocols that incorporate ionizing radiation in the processing of fresh vegetables, product formulation, e.g. proportion of leaf midrib material, will be a key factor in the successful implementation of this antimicrobial intervention.